

REPORT

for

UNITED STATES LETTERS PATENT

on

METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

by

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METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

This invention relates generally to the field of immunotherapy and, more specifically, to methods of stimulating an immune response to cholesteryl ester transfer protein (CETP).

BACKGROUND OF THE INVENTION

Blood cholesterol levels have long been thought to correlate directly with risk of atherosclerotic cardiac disease, the leading cause of heart attacks. More recently, it has been appreciated that blood cholesterol is actually composed of two primary forms: the high density lipoproteins (HDL) and low density lipoproteins (LDL). Rather than being associated with the disease risk, high HDL levels are apparently inversely predictive. In fact, studies have now indicated that HDL has a direct action in protecting against atherosclerosis and may even promote atherosclerosis plaque regression.

Numerous factors are involved in regulating the level of cholesterol in the body. Cholesteryl ester transfer protein (CETP) is an enzyme responsible for transporting cholesterol esters (CE) from HDL to very low density lipoproteins (VLDL) and LDL. VLDL's are eventually converted into LDL. CETP accelerates specifically the exchange of lipid components between pro- and anti-atherogenic lipo protein fractions. In particular, there is a strong inverse correlation between the levels of CETP in the plasma and the levels of HDL cholesterol. CETP activity levels are elevated in individuals suffering from dietary or genetic hypercholesterolemia. Increased levels of CETP activity result in lowered levels of HDL. In contrast, individuals with deficiencies in CETP activity due to mutations in the CETP gene have markedly elevated HDL levels.

Because of HDL'S potentially beneficial effect
in preventing atherosclerosis, there exists a need for
methods which can be used to increase its level in the
serum. Such methods should ideally be specific and
reliable and involve as little invasion of the body as
possible. The present invention satisfies this need and
provides related advantages as well.

The present invention provides a method for increasing HDL cholesterol in a mammal by stimulating an immune response that inhibits the function of CETP. Such an immune response can be induced by immunizing with CETP or fragments of CETP (together termed "CETP Peptides") which contain an epitope capable of stimulating such a response. The peptides can be conjugated to a carrier, such as Keyhole Limpet Hemocyanin (KLH) or ovalbumin, in

order to increase immunogenicity. Adjuvants can also be administered.

In one embodiment, the fragments of CETP used to raise the antibody response are about ten to twenty amino acids in length and contain sequences homologous to the sequence in rabbit or human CETP.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a means to utilize the body's own immune system to lower CETP levels, thereby increasing the level of beneficial HDL cholesterol. The invention provides an effective method of raising HDL in the blood or more specifically, the serum. By utilizing the body's own immune system to increase HDL levels, the invention avoids the problems associated with the repeated administration of drugs, which have undesirable side effects.

According to the present invention, CETP peptide is administered to an appropriate individual in such a manner as to elicit an anti-CETP immune response. The CETP can be chosen to contain an epitope capable of stimulating an antibody or humoral response. Alternatively, the CETP can stimulate a cellular response, or other immune response. CETP peptides can be elected to contain B cell epitopes, sequences capable of stimulating the production of antibodies that specifically recognize and bind to the epitope. Alternatively, CETP peptides can be chosen which stimulate a T cell or more general immune response.

Individuals exhibiting, or at risk of exhibiting, low serum levels of HDL cholesterol are particularly appropriate for such treatment. Serum HDL levels can be determined using methods well-known in the

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Administration of CEST peptide immunogen

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H-Cys-Asp-Ser-Gly-Arg-Val-Arg-Thr-Asp-Ala-Pro-Asp-OH
(SEQ ID No.: 1)

30 H-Cys-Asp-Ala-Gly-Ser-Val-Arg-Thr-Asn-Ala-Pro-Asp-OH
(SEQ ID No.: 2)

H-His-Leu-Leu-Val-Asp-Phe-Leu-Gln-Ser-Leu-Ser-OH.
(SEQ ID No.: 3)

The first peptide (SEQ ID 1) is taken from the Human CETP peptide sequence (residues 131-142 without signal peptide) from Smith and Barakat, Med. Sci. Res., 21:911-912 (1993), which is incorporated herein by reference. The second peptide (SEQ ID 2) is the corresponding rabbit sequence and differs by only 3 amino acids from the human.

The third peptide (SEQ ID 3) is common to both human and rabbit and is an epitope recognized by anti-CETP-monoclonal antibody which is neutralizing. Tall, A.R., J. Lipids Res., 34:1255-1257 (1993).

The peptides were conjugated to ovalbumin by the procedure of Current Protocols in Molecular Biology, supra. Of four New Zealand White rabbits, approximately four months of age, two were injected intramuscularly with 100 micrograms of the ovalbumin-conjugated human peptide (Seq. ID No.: 1) and CFA in PBS saline and two were injected with the equivalent human/rabbit peptide (Seq. ID No. 3). The animals were boosted twice at one month intervals with with the same peptides in IFA..

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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